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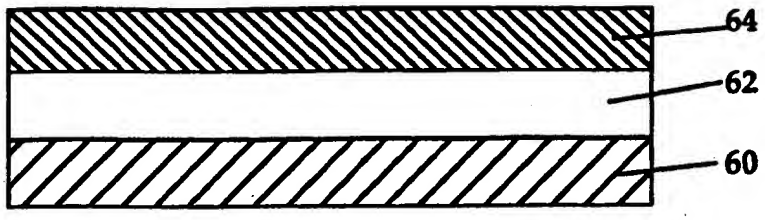
(54) Title: MICROFABRICATED DEVICES FOR DIAGNOSTIC APPLICATIONS

(57) Abstract

A suspension of microfabricated particles, or microdevices, for use in diagnostic applications is disclosed. The microdevices have a selected shape, and uniform dimensions preferably in the 100 nm to 10 μm range. The microdevices can be designed for intravenous or intraoperative administration. Also disclosed are microfabrication methods for making such microdevices.



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MICROFABRICATED DEVICES FOR DIAGNOSTIC APPLICATIONS

Field of the Invention

The present invention relates generally to microfabricated devices, particularly to a suspension of such microdevices useful for diagnostic applications, and to methods of forming such microdevices.

Background of the Invention

Molecular markers and probes bearing such markers are widely used in a variety of chemical, biological and medical applications. One application of this technology is cell sorting in laboratory cultures. The two principal cell sorting methods are through the use of a fluorescence activated cell sorter (FACS), and affinity chromatography. Both methods are applied to suspensions of free floating cells. The FACS method employs labeled chemical probes, such as fluorescently tagged antibodies. Cells are sorted on the basis of their bound fluorescent markers. Affinity chromatography employs a column packed with glass or plastic beads coated with antibodies which bind specific cell marker molecules. Suspended cells are run through the column. Those cells possessing the marker bound by the antibody adhere to the beads. All other cells are washed away, and the bound cells are then isolated.

Molecular marker technology is also used for *in vitro* diagnostics. For instance, U.S. Patent No. 4,219,335 by R.C. Ebersole teaches the use of immune reagents tagged with particles capable of affecting the electrical reactance of a substrate for the *in vitro* detection of immunochemically reactive substances in biological fluids. Colloidal gold particles with a bonded layer of monoclonal antibodies have been used to identify particular molecular markers in tissue cross-sections. The molecules of interest are visualized by electron microscopy by virtue of their attached probes.

Radioactively tagged antibodies are also used for the detection of primary and metastatic tumors. For this application, labeled antibodies injected in the general circulation adhere preferentially to cancerous tissue. γ-ray cameras are then used to detect the more pronouncedly radioactive locations in the body, and those locations are identified as potential metastatic domains. This method permits the identification of a significant share of the metastases and primary tumors of dimensions larger than approximately one millimeter. Unfortunately, all smaller foci are not detected, and thus may not be treated, leading to possible recurrence of the disease or growth and further proliferation of the metastatic domains. Sub-millimeter cancers are not usually detectable with any presently available diagnostic technologies, such as X-rays, ultrasound, and magnetic resonance

imaging (MRI). Currently the most commonly used diagnostic methods are X-ray and ultrasound, despite the fact that it may take a cancer site many years to grow to a size that may be detected by either method. Thus, there is a need for a diagnostic method capable of detecting sub-millimeter neoplasms.

Radioactively tagged antibodies for intra-operative cancer diagnostics are currently in Phase III clinical trials. These are used for localization of small sized metastatic sites in the course of surgery. A suspension of antibodies tagged with a radioactive iodine is applied to tissue suspected of containing metastatic deposits. Radioactivity is detected with a surgeon-operated Geiger counter. Difficulties associated with this method include instrument handling inconvenience for the surgeon, problems localizing the exact masses to be excised, and the inconvenience of handling radiolabeled probes.

Lymphokinase activated killer (LAK) cells bind marker molecules present on tumor capillary walls. When there is a sufficient density of marker molecules, bound LAK cells may block the flow of blood through a capillary. While LAK cells appear to be potentially useful in the treatment of tumors, in practice it has proved difficult to obtain enough LAK cells with sufficiently predictable behavior toward a given tumor type in an individual.

It would be useful to create a device able to predictably recognize and bind a tumor marker so as to block blood supply to the tumor, thereby inhibiting tumor growth. Such a useful device would need to travel freely through the vascular system and predictably bind a preselected tumor-specific marker.

Summary of the Invention

The present invention provides a microstructural device useful for identification and binding of specific molecular markers, comprising a microfabricated structural material, a coating on a portion of the structural material providing aqueous suspension properties, and a coating on another portion of the structural material providing specific molecular binding properties. The structural material may be modified, and additional material components and ligands added, so as to obtain surfaces with the desired heterogeneous population of molecules proving binding to targeted molecules.

Also provided are methods of making microstructural devices for identification and binding of specific molecular markers, comprising depositing a sacrificial layer on a substrate, and generating a layer of a structural material on the sacrificial layer. The structural material is then patterned and etched with desired sizes and shapes of said device. Additional partial or through-thickness heterogeneities may be generated in the devices.

The sacrificial layer is dissolved to release the microdevice structural material from the substrate. Finally, parts of the structural material are coated with a molecular layer which provides aqueous suspension properties, and one or more ligands to specific targeted molecular markers.

The invention provides methods of using the microstructural devices for identification and localization of specific molecular markers and cells bearing such markers, comprising contacting a suspension of the detectable microstructural device coated with a pre-selected ligand with a biological sample, removing the unbound device, and detecting the bound device, wherein detection of the bound device identifies the presence of the molecular marker of interest and/or cells bearing such markers. In a specific embodiment, the biological sample is tissue, and the bound device identifies the location of the molecular marker of interest. Methods for the *in vitro*, intra-operative, and *in vivo* detection of pathologies such as cancer are provided.

The invention further provides therapeutic methods for treating a tumor by binding the device of the invention to tumor-specific antigens expressed in the tumor vasculature such that an embolizing barrier to further blood flow forms, thus inhibiting tumor growth and/or causing tumor death.

In another aspect, the invention includes a suspension of microdevices for use in targeting selected target cells or tissue with a diagnostic agent. The devices are preferably bioerodable microdevices suspended in an aqueous medium, and (i) have a selected non-spherical shape and uniform microdevice dimensions and (ii) contain the diagnostic agent.

In one general embodiment, the microdevices have surface-bound, marker-binding molecules effective to bind to a marker carried on the surface of such target cells or tissue. The suspension may be used, for use in targeting selected cells or tissue via the bloodstream, wherein the microdevices are coated with a hydrophilic polymer, such as a natural or synthetic glycocalyx, effective to enhance maintenance of the microdevices in suspension. The hydrophilic polymer may further be conjugated to vesicle-forming lipids, and the microdevices may be coated with a lipid film containing such vesicle-forming lipids conjugated to the hydrophilic polymer. In a specific embodiment, the marker-binding molecules are (i) bound to the free ends of at least a portion of the hydrophilic polymer, and (ii) effective to bind to a marker carried on the surface of such target cells or tissue.

In another general embodiment, the suspension of microdevices is parenterally administered to a subject, and the microdevices have a selected maximum dimension in the

range between 0.1 and 3 microns. When such a suspension is used for solid-tumor targeting, the microdevices preferably have a maximum dimension less than about 150 nm.

In still another general embodiment, the diagnostic agent contained in the above-described microdevices is released from the microdevices upon microdevice bioerosion. For example, the microdevices may be formed of (or may contain as one of their structural elements) a biodegradable material, a material designed to dissolve in body fluid at a selected dissolution rate, or from a condensed-phase polymer material effective to decondense, at a selected rate, when exposed to plasma. In a specific embodiment, such microdevices formed of a material designed to dissolve in body fluid at a selected dissolution rate are formed of iron, titanium, gold, silver, platinum, copper, aluminum, alloys thereof or oxides thereof.

The microdevices described above are, in one general embodiment, substantially disc-shaped with a laminated structure containing first and second disc-shaped layers formed of first and second different materials, respectively. In one embodiment, the first and second layers have different densities, and a combined density similar to that of the aqueous medium. In another embodiment, where the devices are for use in parenteral administration to a subject, the first and second layers have different rates of erosion in body fluid plasma. In a related embodiment, the microdevices have a trilaminate structure composed of an interior layer sandwiched between a pair of exterior coating layers. The coating layers may have, for example, a slower rate of bioerosion than the interior layer.

In applications where the microdevices are injected intravenously for use in detecting the presence of target cells in a subject, one general embodiment includes microdevices that (i) are effective to circulate over a selected (*e.g.*, extended) period in the bloodstream, and (ii) contain a diagnostic agent effective for detection by X-radiation, scintigraphy, nuclear magnetic resonance, or ultrasound. Microdevices containing such a diagnostic agent may also be administered intraoperatively to detect the presence of target cells in a subject.

The invention further includes, in another aspect, a microfabrication method for producing microdevices for use in the suspensions described above. The method includes (i) forming a sacrificial layer on a substrate layer, (ii) forming a microdevice-structure layer on the sacrificial layer, (iii) lithographically patterning the microdevice-structure layer with a desired microdevice size and shape, (iv) etching the microdevice-structure layer through to the sacrificial layer according to the lithographic pattern produced by the lithographic patterning, and (v) dissolving the sacrificial layer to release the microdevices from the

substrate. In a related aspect, the invention includes a microfabrication method for producing microdevices for use in the suspensions described above. The method includes (i) exposing a sheet of microdevice material to a photoablating light source through a photomask, (ii) by such exposing, forming a reticular lattice pattern on the sheet corresponding to the desired microdevice size and shape, and (iii) continuing the exposure until the desired microdevices are formed.

These and other objects and features of the invention will be further understood when read in connection with the detailed description of the invention and the accompanying drawings.

Brief Description of the Drawing

The accompanying drawings, which are incorporated in and constitute a part of the specification, schematically illustrate a preferred embodiment of the invention and, together with the general description given above and the detailed description of the preferred embodiment given below, serve to explain the principles of the invention.

Figs. 1-9 are schematic sectional views of the steps in the fabrication method of embodiments of the present invention shown in Figures 10-20.

Figures 10 and 11 are schematic plan and sectional views, respectively, of microstructural molecular probes according to the present invention.

Figures 12-15 are schematic sectional views of the binding of microstructural molecular probes according to the present invention to a target region of a capillary wall.

Figures 16-20 are schematic sectional views of three different embodiments of microstructural probe devices according to the present invention and their *in vivo* application.

Figures 21, 22 and 23-33 are schematic perspective, elevational and sectional views, respectively, illustrating various device-target and device-device binding strategies according to the present invention.

Figure 34 is a schematic perspective view of a tumor whose peripheral capillaries have been embolized with devices according to the present invention.

Description of the Preferred Embodiments

As used in this specification and the appended claims, the singular forms "a", "an", and "the" include plural references unless the context clearly indicates otherwise. Thus, for

exampl , reference to "a microstructural device" includes suspensions of such devices, reference to "the method" includes methods and/or steps of the type described herein and/or which will become apparent to those skilled in the art upon reading this disclosure.

The present invention provides a novel microdevice that is useful diagnostically and therapeutically in a variety of *in vitro*, intra-operative, and *in vivo* applications. Binding of the microdevice to the marker results from the specific interaction between one or more ligands coating a portion of the microdevice with the pre-selected molecular marker, such as those occurring in antibody/antigen, enzyme/substrate, lectin/saccharide, and receptor/ligand interactions. Identification of the pre-selected molecular marker results from detection of the bound microdevice.

The terms "microstructural device", "microdevice", or "device" as used in this disclosure are meant to be equivalent and interchangeable, and refer to the microdevice or a suspension of the microdevice of the invention. The "microdevice" has at least one dimension greater than about 0.10 microns and is less than 15 microns in any dimension. A device may have any shape, *e.g.*, circle, square, sphere, cylinder etc. but is most preferably in a disk shape with a diameter of 0.10 to 15 microns and a thickness of about 0.01 to 2 microns. The size and shape is such that the microdevice can move through blood vessels of a mammal (preferably a human) and a plurality of microdevices linked together will block a blood vessel and prevent blood from circulating to a cancer tumor.

The term "molecular coating" is used herein to describe a coating which is bound to the surface of a "microdevice". The molecular coating may be bound directly to the surface of the device or bound to a resin such as an electron donating group, *e.g.*, $-NH_2$, OH or the like derivatized onto the surface of a polymeric material of the device. The molecular coating may encompass all or a portion of the surface area of the device and preferably encompasses all external areas of the device. The molecular coating is characterized by its ability to prevent an immune response from being generated by the coating or microdevice when the device is placed in a mammal *e.g.*, a human. The molecular coating is also characterized by its ability to bind specifically to a naturally occurring biological molecule. Thus, the molecular coating may comprise a natural ligand or portion thereof or an antibody which may be a humanized antibody or a monoclonal antibody. The molecular coating is connectable to or connected to a detectable label which label may be detected visually or by X-rays.

The microdevice of the invention provide advantages over prior art marker technology and natural substances such as labeled antibodies. For example, fabrication

methods and materials may be varied to achieve a microdevice with one or more highly specific characteristics and functionalities. The specific structural materials used in fabrication of the microdevice can be altered depending on the desired use and required characteristics of the microdevice. Structural materials are selected from a vast array of those available, including metals, alloys, polymers, bioresorbable materials, and semiconductors, and afford the ability to engineer the shape of the microdevice according to specific design criteria. For instance, the device may be formed as a thin disk so as to maximize the surface area to volume ratio, and thus increase its adherence to vascular walls. Further, the microfabricated structures can be partially coated with an agent to improve its suspension in aqueous liquids. The microdevice may be composed of several different layers of specified thickness, including in the sub-micron range. The location and relative proportion of one or more functionalities may also be varied to achieve the desired optimum for a specific application.

The microdevice according to the present invention may be fabricated to have a controlled lifetime in the environment in which it is designed to function. Depending on the application, it may be designed to dissolve in the bloodstream within an hour, or to last for a month, for example. It may be biodegradable by means of enzymes naturally present in the body, for instance if made of high molecular weight glycogen or collagen, or chemically soluble, for instance, if made of iron or zinc. A preferred embodiment of the present invention uses permanent structural material made of biologically inert silicon. Other materials, such as gold, iridium, polyethylene, or bioresorbable materials may also be used.

There are several requirements for functionality and survivability of the microdevice in the vascular system. First, it needs to be small enough to be carried through capillaries unhindered. Second, it preferably has a molecular coating that which does not elicit a rapid immune response in the recipient subject. The immune system does not respond to the fundamental molecules required for vertebrate life such as glycogen, RNA, and collagen, compositionally simple molecules such as polyglycine, or any polypeptide consisting of a single type of amino acid. The immune system also does not respond to most polypeptides composed of dextro-rotary amino acids since all living species use only levo-rotary amino acids. In addition, the immune system generally does not respond strongly to molecules that are only slightly different from self molecules.

Examples of how to evade the immune system are provided by certain naturally occurring antigens. For example the parasitic trypanosome that causes sleeping sickness is covered by a compositionally simple coat of a single molecular species of glycoprotein.

Even though the molecule is different from host self molecules, it takes over on week for the immune response to develop.

Parasites that carry many types of molecules on their surface are engaged by many different antibodies in the blood of the host and produce a strong immune response more rapidly. By following the strategy of coating the injected structures with molecules that are largely ignored by the immune system, devices according to the present invention are able to circulate for at least one week.

Erythrocytes (red blood cells) have an outer coating called the glycocalyx which consists of hydrophilic chains of glycoproteins, such as glycophorin, and glycolipids over 1000 Å long. These have a hydrophobic section which is anchored in the hydrophobic lipid layer of the cell membrane, while the hydrophilic chain extends out into the aqueous environment. This keeps the cell suspended in the blood. An artificial mechanical structure of similar size and density, if coated with a synthetic glycocalyx, would be carried by the blood just like an erythrocyte. Hydrophilic natural polymers such as glycogen or polyserine are examples of suitable molecules for this purpose which are employed by embodiments of the present invention.

The microdevice of the invention is useful to identify and/or locate a pre-selected molecular marker, antigen, cell, or the presence of a molecular marker, antigen, or cell in a biological sample. Such a marker may be expressed by a variety of systems, including cells, viruses, bacteria, and multi-cellular parasites. Synthetic structures according to the present invention can be made at any of these size scales and placed in the circulatory system to perform desired functions.

Cells and molecules floating in the blood stream interact on the basis of non-covalent binding forces such as hydrophobic, Van der Waals, and electrostatic forces. Lock and key specificity is achieved in natural systems by controlling the three dimensional arrangement of small hydrophobic, hydrophilic, positively charged, and negatively charged groups. The present state of the art in biochemistry provides the ability to fabricate specific desired molecular arrangements that may be used as coatings for the devices of the present invention in order to target them to molecules or molecular arrangements specific to cells or tissues of interest. A preferred embodiment of the present invention for *in vivo* applications provides a microfabricated platform of appropriate size and shape that carries a community of cooperating molecules and maintains them in a state of organization such that, as a group, they can perform jobs that are impossible for natural molecules.

In general terms, microdevices according to the above-described embodiments of the present invention may be fabricated as follows. As shown in Figure 1, a substrate 60, such as a silicon wafer, is coated with a sacrificial layer 62, such as phosphorous doped silicon dioxide, deposited by chemical vapor deposition. The sacrificial layer is coated with a structural material 64 such as polycrystalline silicon by chemical vapor deposition. As shown in Figure 2, the structural material 64 is then lithographically patterned and etched according to any of the processes well known in the art to define the sizes and shapes of the desired structures, *e.g.*, photolithography, electron beam lithography, and X-ray lithography. Additional details on microfabrication methods useful in the manufacture of devices according to the present invention are described in commonly-assigned, co-pending PCT patent publications WO 95/24261, WO 95/24472 and WO 95/24736.

One or more additional coatings 66, such as polystyrene, may be also be applied, as shown in Figure 3, and then subjected to an anisotropic blanket etch that removes this coating 66 from all horizontal surfaces to leave only the vertical sidewalls 68, as shown in Figure 4. Chemical etching of the sacrificial layer 62 releases the structure 70, shown in Figure 5, with a chemically different material on its faces 72 and edges 68. Chemically different areas simplify the attachment of different chemical species using different chemical coupling groups. Thus the edge 68 may be coated with long chain hydrophilic molecules, such as a surfactant, for suspension in aqueous media, while the faces 72 may be coated with antibodies or ligands that bind to the marker molecules in target cells of interest.

Figures 6-9 show how the same method may be used to fabricate structures 80 having different mechanical features, by molding the substrate to produce the desired topography such as a depression or cavity 74, for instance, by etching.

Methods of coating inorganic substrates with surfactants are well known in the art. Coating of the devices with antibodies and other ligands to pre-selected marker molecules may also be achieved by methods known in the art. Such methods are described, for example, in Taylor, R., ed., Protein Immobilization Fundamentals and Applications (1991), at 109-110. For example, when the device is coated with a target-specific antibody, the terminal carboxylic acid group of the Fc region of an antibody is covalently bonded to a linking molecule, which in turn is covalently bonded to the device structure. The linking molecule should be a chain of at least 5 carbons so that it is flexible enough not to distort the antibody and affect its activity. An example of a linking molecule useful in the present invention is an organochlorosilane. Preferred linking molecules are 3-amino-propyl triethoxy silane (APTES), glycidoxypropyl trimethoxy silane, vinyl, methacrylate, epoxy,

amine and mercaptan-functional silanes. The region of the antibody that binds to the linking molecule is different from that region that binds to the target antigen. Thus, the operations of surface immobilization and target interaction are not mutually competitive, and the immobilized ligand is effective for interacting with its target marker molecule. In one embodiment, a covalent peptide bond is formed between the linking molecule and the carboxylic acid terminal group of the antibody.

The devices can be fabricated with materials that are easily detectable. For example, in various specific embodiments, embodiment the device is fabricated with material resolvable by X-ray, MRI, nuclear magnetic resonance (NMR), or ultrasound. X-ray-resolvable materials include iron, silicon, gold and gadolinum. MRI-resolvable materials include gadolinum and iron.

The microdevices can be tagged so as to allow detection or visualization. For example, microdevices are rendered radioactive by implantation or surface attachment of radioactive isotopes such as I-123, I-125, I-131, In-111 and Tc-99m. Radioactive devices concentrated at a site of disease can be identified by a radiation detectors such as the γ-ray cameras currently used in scintigraphy (bone scans), resulting in identification and localization of such regions. Microdevices can also be tagged with fluorescent molecules or dyes, such that a concentration of microdevices can be detected visually.

According to the invention, antibodies or other specific ligands are covalently bound to the microstructural device, such that the bond is stronger than the non-covalent bond between the antibody and the target molecule, ensuring that the device remains bound to the target molecular marker. Covalent attachment may be accomplished in a number of ways known to the art, for example, oxidation of the surface of the device, followed by silanization and use of a homogeneous bifunctional linker molecule, such as glutaraldehyde.

Antibodies or ligands to disease-associated molecules can be used to coat the microdevice structural material of the present invention for purposes of targeting the device to those specific diseased cells. The cell membranes of cancer cells are generally found to have a disproportionately high population of lectins, tri- and tetra-antennary oligosaccharides with GlcNac(β-1,6) and Man(α-1,6) branch points, certain sphingolipids, and sialic acid. The tumor autocrine mobility factor (TAMF), found in breast cancer cells, stimulates the extension of pseudopodia that are instrumental to the initiation of the metastatic cascade. A similar function is performed by the tumor angiogenesis factor (TAF). Depending on the type of cancer or in the case of other pathologies and disorder,

other types of molecules may also have abnormally high population on the membrane surface.

This invention is not restricted to the use of a specific antibody or ligand. By the term "antibody" or "ligand" is meant any agent which can interact specifically with a pre-selected molecular marker so as to identify and bind the pre-selected molecular marker, including natural and synthetic antibodies, protein, polypeptide, peptide, carbohydrate, oligonucleotides, glycoproteins, glycolipids, and the like. The term "antibody" includes intact molecules as well as fragments thereof, such as Fab, $F(ab')_2$, and Fv which are capable of binding the epitopic determinant of the molecular marker of interest. Antibodies can be polyclonal or monoclonal antibodies, or fragments thereof. It is noted that a large number of monoclonal antibodies to tumor-specific antigens are known to the art. See, for example, pages 301-323 of Cancer, 3d Ed., edited by V. T De Vita, S. Hellmann, and S.A. Rosenberg. Among the specific anti-tumor antibodies known are those that bind to pancarcinoma antigens (PCAs), an example of which is the tumor-associated glycoprotein antigen known as TAG-72. Such antibodies thus bind to a large spectrum of cancers, and to different cellular mutations in the metastatic cascade. Examples of antibodies of this type are B-72.3 and the CC49 antibodies. Microdevices coated with anti-PCA antibodies are particularly useful in the diagnosis and treatment of different neoplasms, and of the metastatic proliferation of a primary tumor. Retention of microdevices in the tumor can be improved by using microdevices less than about 150 nm in diameter. Such small microdevices can migrate through the ~150 nm pores present in capillaries supplying tumors, enabling the devices to concentrate more effectively in the tumor and thus provide a stronger diagnostic signal from the tumor.

Any molecular marker of interest may be detected by the methods of the invention so long as the device is fabricated with a marker-specific ligand or antibody. Preferably, the molecular markers to be identified are tumor-specific antigens. In selecting specific antibodies for *in vivo* applications, an efficient strategy is to use human antibodies specific for the common generic molecules that are relatively more plentiful in target diseased tissue cell membranes than in healthy cell membranes. The selection of an antibody or ligand for detecting the presence of a specific disease-associated marker, and hence the presence of disease, or for therapeutic treatment of disease, as well as the method of detecting the bound device is determined by one skilled in the art based on a number of factors, including availability of ligands, specificity of available ligands, placement in the body, etc. In a

specific situation, detection or treatment methods may involve administration of microstructural devices having a combination of pre-selected ligands.

When it is desirable to detect a disease which is not associated with a specific molecular marker, other strategies may be utilized. For example, if a disease condition is associated with a different proportion of two or more marker molecules that are expressed in healthy cells or tissues, the detection method of the invention is altered to contact the biological sample to be tested with a series of coated ligands carrying varying tags. For example, if malignancy X is associated with expression of 75% marker 1 and 25% marker 2, and normal tissue expression is 10% marker 1 and 90% marker 2. the biological sample is sequentially contacted with two types of devices, one carrying a marker 1-specific ligand (tagged with a red fluorescing molecule) and a marker 2-specific ligand (tagged with a blue fluorescing molecule). The disease-associated alteration of marker expression is thus detected by examining the resulting fluorescence.

The microdevice of the invention can be coated with a variety of ligands, including those commercially available or generated by methods well known to the art. For example, antibodies and fragments thereof may be generated by methods such as those described by Kohler et al. (1975) Nature 256:495. See also, Harlow & Lane (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. Specific binding polypeptides may be generated to pre-selected target cells and molecular markers be methods known in the art, including generation of peptide libraries and a lithographic method. For example, U.S. Patent No. 5,010,175 teaches a method for generating a mixture of peptides selectable for a specific property, *e.g.*, high-specificity binding to a target molecule. See also, U.S. Patent No. 4,833,092 and U.S. Patent No. 5,194,392. The target molecule in one embodiment of the invention is a tumor-specific protein expressed in the surface of tumor capillary vessels. In preparing binding groups for a photolithographically patterned silicon wafer, the polypeptides are grown on the ends of the same linking chain molecule that will be used on the device so that the binding function will work *in vivo* as tested *in vitro*. The chains are a standard hydrophilic molecule that does not arouse a rejection response from the immune system. Linear polyvinyl alcohol, or polyserine, are examples of preferred candidates. High-specificity polynucleotide ligands may be generated to pre-selected marker molecules by such methods as those described by Tuerk & Gold (1990) Science 249:505-510.

The microstructural device of the invention may be used *in vitro* or *in vivo* to detect the presence of a molecular marker of interest. The invention provides a method for

detecting a pre-selected molecular marker consisting of contacting the microstructural device coated with a ligand specific to the molecular marker of interest with a biological sample, and detecting binding of the device to the molecular marker. By the term "biological sample" is meant any fluid, cell or tissue sample obtained from a subject in which it is desirable to test for the presence of a molecular marker of interest, including blood, plasma, urine, cerebrospinal fluid, cells, tissues, or organs. Preferably the subject is human.

In a specific embodiment of the *in vitro* diagnostic method of the invention, the device of the invention is coated with monoclonal antibodies specific to surface markers or cytoplasmic determinants of cells associated with a particular disease or medical disorder. A suspension is formed with the coated devices, and applied to a biological sample, such as a physiological fluid, cell, tissue biopsies, etc. Direct or automated microscopic observation follows, after washing of the tissue, to locate the stably bound microdevices and count their surface density. An abnormal concentration of stably bound devices may be used as an indicator of disease or of the presence of significant prognostic factors. The same method can be applied to blood samples, in order to determine and classify leukemia and lymphomas. Antigens to be targeted for this application include cytoplasmic or surface immunoglobulins, T-cell antigens, and the common acute lymphocytic leukemia antigen (CALLA). The described method offers increased sensitivity for the detection of neoplastic cells in aspirates of hematopoietic cells from marrow, without making recourse to complex or expensive genetic methods such as polymerase chain reaction (PCR).

The concentration of the suspension of the device to be used depends on a number of factors, including the method used to detect the bound device. For example, devices may be tagged with visually detectable dyes or fluorescent molecules, radioactive molecules, or contain X-ray detectable material. The microdevices may also be fabricated into clearly identifiable shapes to aid visualization, *e.g.*, squares, rectangles, circles, etc.

The described method may be similarly applied to urine samples in order to determine the presence of bladder cancer, or as an alternative to cervical and vaginal cytology. The detection method of the invention offers several advantages over conventional histopathology methods, including effectiveness in areas where histology is problematic, as is the case, for instance, in the diagnosis of B-cell lymphomas. For such application, excessive concentration of cells expressing of k or lambda light chains are detected by employing devices coated with specific anti-k or lambda light chain monoclonal antibodies.

The detection method of the invention can also be used as an intra-operative diagnostic procedure, that is for diagnostic assessments performed during the course of a surgical procedure. For example, the method can be used during the surgical excision of a solid tumor such as a primary cancer of the stomach with diffuse microscopic metastases, or a carcinoma of the uterine cervix that may have generated a metastatic involvement of the pelvic wall. When metastatic lesions are too small to be visually detectable, the risk is present that a metastatic deposit will be left *in situ*. The intra-operative detection method of the invention provides a simple, rapid and efficient method of probing the surrounding tissue to guide surgical decisions. In this embodiment, a suspension of microstructural devices coated with ligand specific to the tumor-specific antigen of interest, and the suspension is applied to the area surrounding a visually-detectable tumor or neoplastic lesion. The binding of the device to the tissue can be detected in a number of ways, including by X-ray, color, fluorescence, radioactivity, etc. Observation of binding indicates the presence of microscopic metastatic domains, which may then be removed.

In a specific embodiment, the invention provides a method for the *in vivo* detection of tumors by coating the device of the invention with antibodies or ligands specific to tumor vasculature. Antibodies are selected that target antigens expressed solely or preferentially on tumor capillary walls, and the antibody-coated devices are administered to the recipient subject. Changes in the expression of components of the microcirculatory walls are known to be associated with neoplastic pathologies, and thus can be used as target markers for the intravascular microdevice of the invention. For example, exposed subendothelial basement membrane is overexpressed in the tumor circulatory system, and thus anti-collagen IV antibodies administered to a patient would preferentially bind within neoplastic lesions. The size of the tumor is not a determinant factor in the identification of the lesion, in that it is the microdevice clusters that are detected, not the tumor itself. Thus, neoplastic deposits of any dimension can be identified, including submillimeter lesions. By contrast, current clinical detection limits for neoplastic lesions are in the millimeter range.

For *in vivo* detection of binding to blood vessel targets, binding between the device and molecular marker needs to be sufficiently strong to overcome the blood flow force. For binding of the device to a marker expressed on the surface of a blood vessel wall, the design of the microstructural device can be modified to maximize device-marker binding. For example, in this embodiment the device is fabricated so as to be flexible such that the device can bend to the shape of capillary wall. Alternatively, the device may deform the capillary to its shape, to maximize contact and thus adhesive strength.

The invention provides a method for *in vivo* therapeutic treatment of a disease or disorder, by administration of an effective amount of the device of the invention coated with a disease-specific antibody or ligand, such that the device binds to the cell of interest in sufficient concentration to inhibit or ameliorate the disease being treated. In one embodiment, the microdevice binds to the vasculature of the target tissue periphery in such a manner as to result in restriction or blockage of the blood flow supplying the tissue, and thereby render it hypoxic and eventually causing tissue death or impairment of growth. If the adhesive force available is strong enough, a single device may be sufficient. If not, the goal of embolization may be reached in two or more steps using different structures. Thus, the microdevices remain in the targeted vessel, the vessel is effectively occluded, and re-establishment of the breached vessel does not occur.

Administration of the device to a recipient subject for *in vivo* diagnostic and/or therapeutic purposes may be systemic or local. Administered antibody-coated devices travel freely throughout the circulatory system until they bind to their corresponding antigen. The method used to introduce a suspension of the device will be any convenient method normally used to introduce pharmaceuticals into the bloodstream. Systemic administration may be achieved by injection in the general circulation, either by trickle-feed i.v., bolus, infusion, or the like. The devices can also be administered locally to the vicinity of a known lesion by use of guided catheter system, such as a CAT-scan guided catheter. The concentration of the suspension of the device to be administered which will be effective for the treatment of the disease or disorder being treated will depend on a number of factors, including the particular recipient and the medical condition being treated. Generally, a dose equal to 2% of total blood volume is well tolerated by a patient. If the devices comprise 50% of the volume injected (*e.g.*, 50% v/v), then 1% of the total blood volume will be occupied by the devices. Thus in any given capillary for every microliter of blood that goes through there will be 10 nanoliters of devices. This is enough to coat the wall of a capillary in a tumor. Smaller doses could be used, and proportionately longer times allowed for treatment to occur.

Generally, the suspension of the device to be administered can be formulated with a pharmaceutically acceptable carrier. An acceptable pharmaceutical carrier is one that is non-toxic to the recipient at the dosages and concentrations employed and is compatible with other ingredients in the formulation. Preferably, the suspension is composed of a given concentration of device and a solution which is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, dextrose

solution, and 5% human serum albumin. Nonaqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes. Generally, the carrier can contain minor amounts of additives such as substances that enhance isotonicity and chemical stability, *e.g.*, buffers and preservatives, as well as low molecular weight (less than about 10 residues) polypeptides, proteins, amino acids, carbohydrates including glucose or dextrans, chelating agents such as EDTA, or other excipients.

Figures 10 and 11 show a specific embodiment of the present invention, that is appropriate for intravascular use. It consists of a thin disk 100 with diameter D between about 0.10 to 5 microns and thickness T between about 0.01 and 2 microns. In the embodiment shown in Figures 10-12, the body 102 may be fabricated of polysilicon according to the methods described with reference to Figures 1-9. Devices comprised of soluble material, such as iron or zinc, that will ultimately corrode or dissolve in the blood, may also be used. These soluble devices are coated with a corrosion delay layer 104, such as gold or nickel. The thickness of the corrosion delay layer is set to provide the desired lifetime in the bloodstream.

Referring to Figure 11, the corrosion delay layer 104, in the case of a device 100 made of soluble material, or the body 102, in the case of a device 100 made of inert material, is coated with a layer of various molecules 106 that comprise the biochemical interface to the blood. The side 108 of the disk that will bind to the capillary wall is coated with receptor molecules 110, such as IgG antibodies, that specifically bind to the marker molecules on the tumor capillaries. A portion of the disk 100, such as the edge, may additionally be coated with a surfactant layer (not shown), such as a long chain hydrophilic molecule or a biocompatible detergent to improve the suspension characteristics of the device 100. Suitable detergents include non-ionic polymerized oxyethylene ethers, for example, "TRITON-X 100".

The disk 100 is compliant enough to bend out of plane and acquire the arcuate shape needed to achieve substantially full contact with the cylindrical wall of the capillary. This compliance is controlled by varying the thickness of the disk and selecting a material with the appropriate elastic modulus and ductility. The disk is preferably compliant enough that the adhesive force available at the tumor capillary wall will bend it into full contact. The marker molecule does not have to be one that exists exclusively in the tumor capillaries and nowhere else. It merely needs to be present in a significantly greater surface concentration in the tumor capillaries than in the healthy capillaries.

Referring to Figure 12, an important feature of devices according to the present invention is that the total shear strength, represented by arrow 112, of the interface between the device 100 and the diseased capillary wall 114 is greater than the sum of the blood flow forces, represented by arrows 116 and 118, acting to move the device 100 downstream. This provides a mechanical means to effectively distinguish between healthy and diseased tissue represented by the number of marker molecules 120 per unit area on the capillary wall 114. A healthy capillary will not have a sufficient density of marker molecules 120 on its surface to provide the binding force needed to bend the disk 100 and hold it against the drag force 116 and 118 from the blood flow. Thus, binding will only occur where a minimum threshold of marker molecules 120, indicating the target, is present on the capillary wall 114.

The disks 110 circulate throughout the system as inert bodies except at capillary walls 114 in the target tissues. Here there is a sufficient surface density of marker molecules 120 on the inner wall 114 of the capillary, to anchor the disks 100 against the blood flow 116 and 118. Thus the sum of the drag forces 116 and 118 is less than the ultimate shear strength 112 due to the non-covalent bonds between the marker molecules 120 and the receptors 110 of the disk coating 106.

Marker molecules in the cell membrane are free to diffuse laterally over the surface of the membrane. Figures 13-15 show how the initial random distribution of marker molecules 120, that is sufficient to trap a microstructural device according to the present invention, such as the disk 100 described above, on the capillary wall 114 in the first place, evolves to a much stronger bond as more membrane marker molecules 120 encounter the binding receptors 110 on the disk 100 in the course of their random thermal diffusion in the cell membrane of the capillary wall 114.

After sufficient time has passed such that any target tissues present will have their capillaries lined with these anchored disks 100, or other such compliant structures, a second type of structure may be introduced to the circulatory system. Figure 16 shows, in side view cross-section, how, in one embodiment, the second injected structure 122 is long enough to span across several of the first structures 100 so that the total shear strength available to hold the assembly to the capillary wall is greater than the fluid forces from the passing blood. Additional layers of structures 124 may be used to ultimately block the capillary blood flow completely.

The set of bonding molecules attached to the surfaces of the various structures is chosen such that for each type of structure, each surface is treated with a molecule that is

complementary to a specific molecule on one surface of another structure to which it is intended to adhere. In a preferred embodiment, human antibodies are used as binding ligands. Human antibodies such as IgG will not cause a strong immune response in the human patient. For example, the first injected structure may have the surface 126 opposite the capillary binding surface coated with polythreonine which binds to one surface 128 of the second injected structure 122, which is coated with an antibody to polythreonine.

Figure 17 shows the assembly of structures described immediately above in end view cross-section. The purpose of this strategy is to take advantage of a dilute distribution of marker molecules, which provide a very weak force per unit area, by integrating over a large area to get a large binding force.

In addition to the embodiments described above, Figures 18 and 19 show, in end view cross section, another set of structures useful for *in vivo* applications. The first injected structure 130, made of the same materials as the disk embodiment 100 described above, has a coating 132 of, for example, the appropriate antibodies, on its convex surface 134 which will bind to the marker molecules 120 in the capillary walls 114 of the target tissue. The other surfaces have a different coating 136, such as glycogen, to provide suspension in the blood. After the first structures 130 have had enough time to anchor to any diseased capillaries 114, a second structure 138 is injected. As seen in Figure 20, in side view cross section, the second structure 138 is simply a plug that is small enough to travel unhindered through the healthy capillaries, but large enough in diameter to be caught by the first structures 130 anchored to the diseased capillaries 114. As in the previously described arrangement, this system allows the shear strength of many bonds to marker molecules 120 to be added together to ensure that the fluid forces from the blood will be too small to sweep the assembly 130 and 138 away.

Figures 10-12 show an embodiment of the present invention that is also useful for *in vitro* or intra-operative diagnostics. For such applications, the thickness of the disk 100 may be 0.25 to 50 micrometers, and the diameter may be between about 0.25 micrometers and about one centimeter. The larger dimensions help the laboratory analyst or surgeon in the visual identification of the bound microdevices and thus of the cancer cells or tissues. For laboratory or intraoperative purposes, the corrosion coating 104 is not necessary, and the side 108 is coated with antibodies or ligands that will bind to cancer-specific antigens on the suspect cells or tissue. As in the case of the intravascular devices, the design of the structure 100 may be modified to a different, more easily visible plan view, such as a star

or a square. For both *in vitro* and *in vivo* diagnostics, the devices 100 may be rendered fluorescent or radioactively tagged.

Referring to Figures 21-23, details of the noncovalent binding of molecules 150 selected according to one of the methods described above and bound to devices according to the present invention are shown. Molecules 150 covalently bound to the device 152 bind to marker molecules 154 present only on the target 156, or present at a relatively higher density on the target 156, for example, on the walls of diseased capillaries rather than on the walls of healthy capillaries, or to other devices 158. The binding molecules 160 on the nontarget-binding side 162 of the first administered device 152 may not bind to molecules on the target 156. These molecules 160 may bind instead to a molecule 164 in a coating that is on one side 166 of the next administered device 158. Alternatively, the first injected device 152 may have the same coating on both sides, and the second device 158 may be coated with the same composition of marker molecules as the target 156.

To keep the devices 152, 158 suspended in aqueous solution, long hydrophilic molecules 168 may be bonded to the edges 170 of the device structures 152, 158. Other long hydrophilic molecules 172 may be required on the working surfaces 174 and 176 of the devices 152, 158 to ensure proper suspension in the blood. These hydrophilic molecules 172 may also participate in the non-covalent bonding of device 152 to target 156 or other devices 158. For instance, polyserine, and an IgG antibody to polyserine could be used.

In addition, such hydrophilic molecules may be conjugated to vesicle-forming lipids, and the conjugated lipids may then be used to coat microdevices of the present invention. The hydrophilic molecules may additionally contain a marker-binding molecule (*i.e.*, a ligand) at their free end. Such marker-binding molecules may be conjugated to activated free ends of the hydrophilic molecules using known methods. Examples of suitable hydrophilic molecules include polyethylene glycol chains having molecular weights between about 1,000 and 10,000 daltons. Other suitable hydrophilic molecules are polyvinylpyrrolidone (PVP), polymethyloxazoline, polyethyloxazoline, polyhydroxypropyl methacrylamide, polymethacrylamide and polydimethylacrylamide, polylactic acid, polyglycolic acid, and derivatized celluloses, such as hydroxymethylcellulose or hydroxyethylcellulose.

A "vesicle-forming lipid" in this context refers to any lipid capable of forming part of a stable micelle or liposome composition. Such a lipid typically includes one, preferably two hydrophobic acyl hydrocarbon chains or a steroid group and preferably contains a

chemically reactive group, such as an amine, acid, ester, aldehyde or alcohol, at its polar head group. Included in this class are the phospholipids, such as phosphatidylcholine (PC), phosphatidic acid (PA), phosphatidylinositol (PI), and sphingomyelin (SM), where the two hydrocarbon chains are typically between about 14-22 carbon atoms in length, and have varying degrees of unsaturation. The above-described lipids and phospholipids whose acyl chains have a variety of degrees of saturation can be obtained commercially, or prepared according to published methods. An exemplary phospholipid is phosphatidylethanolamine (PE), which provides a reactive amino group which is convenient for coupling to the activated polymers. An exemplary PE is distearyl PE (DSPE). Methods for conjugating polymers such as described above to reactive groups on polar heads of lipids such as described above are well-known.

Figures 24 and 25 show an embodiment of a coating according to the present invention that does not use antibodies to cause two devices according to the present invention to adhere to each other. The molecular species 180 on the surface of one structure 182 has a hydrophobic group 184 which fits well in a hydrophobic pocket 186 of a molecule 188 on a second structure 190. The hydrophobic group 184 is coiled up when in contact with the aqueous environment, but uncoils when in the hydrophobic pocket, as shown in Figure 25. The hydrophobic group 184 may be held out in the aqueous environment by a hydrophilic chain 192. Examples of molecules useful in this embodiment are, for the hydrophilic chain 192, a ten carbon oligomeric unit of vinyl alcohol, for the hydrophobic group 184, a decyl group ($-(CH_2)_9CH_3$), and, for the hydrophobic pocket 188, 15 mers of poly(3-methyl-1-butanol) $(-C(OH)CH(CH(CH_3)_2)-)_{15}CH_3$.

Figures 26 and 27 show another embodiment of the strategy of complimentary molecules to achieve bonding through proximity of groups of similar hydrophobicity or hydrophilicity. The first device 182 has the same coating as described with respect to Figures 24 and 25. Molecules 194 on the surface of the second device 196 provide hydrophobic regions 198 between adjacent molecules 194, rather than the hydrophobic pockets 186 within a single molecule 188, described above. The hydrophobic molecule 194 may be polyvaline, or polyisoleucine.

Figures 28 and 29 show the strategy of using antibodies 200 covalently bound at the end of their Fc region 202 to the surface of a device 204 so that the binding regions 206 are available to bind to the epitopes 208 of the molecule 210 that is the target for this particular antibody 200. Where the target 212 is another device, the target molecule 210 may be covalently attached to the device 212 by means of a flexible hydrophilic chain 214, such as

a 30 angstrom long oligomer of vinyl alcohol. The chain 214 is covalently bound to the device 212 and to a region of the target group 210 that does not interfere with its binding to the antibody 200. Figure 29 shows the bound state of the molecules 200 and 210.

Figure 30 shows how a gradient of increasing hydrophobicity can be established by decreasing the number of hydrophobic groups 220 and 222 and increasing the number of hydrophilic groups 224 per unit volume as a function of increasing distance from the surface 226 of the device 228. Thus a synthetic glycocalyx can be tailored to provide the functional coat desired for a particular device. A hydrophobic environment 230 can be maintained near the surface 226 of the device 228 to harbor mobile hydrophobic groups, while the entire device 228 is kept in suspension in an aqueous environment by hydrophilic groups 224 on long chains.

Figures 31-33 show a mechanism for further securing microstructural devices according to the present invention 240 to the membrane of a target cell 242 more strongly than would occur from binding with the marker molecules 244 alone. The binding surface 246 of the device 240 has binding molecules 248 that make the 5 initial binding of the device 240 to the target cell 242. The device surface 246 may also have attached molecules 250 which have a hydrophilic chain 252, such as polyserine, 50 to 1000 Å long, with a hydrophobic chain 254, such as polyvaline, 30 to 100 angstroms long, at the free end.

In an aqueous environment 255, such as blood, the hydrophobic end 254 spends most of its time coiled up in proximity to the slightly hydrophobic device surface molecular layer 256 to minimize contact with water. The device surface may be methylated, for example, to render it slightly hydrophobic. Random thermal motion causes the hydrophobic ends 254 to intermittently extend out, but finding only a repulsive aqueous environment 255 they quickly return to the predominant coiled conformation. When the device 240 is anchored to a cell wall 242 by marker molecules 244, there is now a hydrophobic region 258 within reach of the hydrophobic ends 254 of the molecules 250. This hydrophobic region 258 is the lipid bilayer of the target cell membranes 242, for instance the epithelial cells that line the capillary walls of a tumor. Each time random thermal motion causes a molecule 250 to extend in the direction of the membrane 242 there is a finite chance that the hydrophobic chain 254 will enter the hydrophobic region 258. This achieves the bound state shown in Figure 32. As seen in detail in Figure 33, the hydrophobic chain 254 uncoils inside the hydrophobic region 258 since the interactions with the environment are now energetically favorable. The hydrophilic section 252 of the molecule 250 remains in the aqueous region 255. When this process occurs, the free energy of the system is

lowered. Therefor , this is the preferred state, and over time many of thes molecules 250 bridge between the device 240 and the membrane 242. This strengthens the force of attachment of the disk to the target cell far beyond what is possible with bonding to marker molecules 244 alone.

Within the vasculature, this process only occurs in the diseased capillaries since that is the only place where the devices spend a significant period of time adjacent to a hydrophobic layer. This provides a secure foundation which can hold a second layer of devices, such as those described with reference to Figure 16-20, above, against the flow of blood.

Figure 34 shows the desired final state wherein an undesirable tissue 300, such as a tumor, seen here as spherical, has accumulated a hemispherical shell of emboli 302 located in the capillaries 304 that bring blood to the tumor 300. The emboli 302 form just at the boundary of the tumor 300 where the tumor-specific molecular markers are first encountered by the incoming microstructures. The minimum quantity of structures that is typically injected is that which ensures formation of a thin impermeable shell blocking all incoming capillaries of each tumor. It would be redundant to also block outgoing capillaries since they are in series with incoming ones. It is not necessary to fill the three dimensional volume of a tumor with devices, only a two dimensional barrier is needed. As a practical matter, it will be necessary to inject more than the theoretical minimum quantity of devices so that the treatment can be accomplished in a shorter period of time.

The devices of the present invention have been described in relation to an intravascular *in vivo* treatment of undesirable tissues, such as tumors. The invention may also be used in the *in vivo* treatment of other conditions where selective local capillary blockage is desirable. To treat a human subject, the subject is injected i.v. with 100 ml of a 50% suspension of microdevices as shown in 100 of Figure 16, or as shown in 130 of Figure 18, having a ligand to a tumor-specific capillary or microvessel wall marker and a marker ligand available for binding to a second microdevice. Sufficient time is allowed for the microdevices to bind to the tumor-specific capillary wall marker, *e.g.*, 15 to 30 min. Approximately 20 ml of a 20% suspension of a second microdevice is administered which consists of conjugate embolizing microdevices such as those of 122 of Figure 16 or those of 138 of Figure 20. The microdevices of the second suspension bind to the microdevices of the first suspension, effectively forming embolizing structures in the tumor capillaries and microvessels, which restrict further blood flow to the tumors. Treatment may be repeated daily until remission is observed.

IT IS CLAIMED:

1. A microstructural device for identification and binding of specific molecular markers, comprising

a microfabricated structure having at least one dimension greater than 0.2 microns, and

at least one molecular coating on said structure providing specific molecular binding properties.

2. A microstructural device of claim 1, wherein said device is from about 0.25 - 15 microns in two dimensions and 0.01 - 2 microns in a third dimension.

3. A microstructural device of claim 2, wherein said device is a disk with a diameter from about 0.25 - 15 microns and a thickness from about 0.01 - 2 microns.

4. A microstructural device of claim 2, wherein said device is a rectangular parallelopiped having a length from about 0.25 - 15 microns, a width from about 0.25 - 5 microns, and a thickness from about 0.01 - 2 microns.

5. A microstructural device of any of claims 1-4, wherein said device is comprised of polysilicon, and said binding coating comprises antibodies.

6. A microstructural device of any of claims 1-5, further comprising molecules bound on said structure providing aqueous suspension properties.

7. A microstructural device of claim 6, wherein said structure is comprised of polysilicon, said binding coating comprises antibodies, and said suspension coating comprises long chain hydrophilic molecules.

8. A microstructural device of any of claims 1-7, wherein said structure has different binding coatings on opposite sides.

9. A microstructural device of claim 8, wherein said structure has a first coating for binding to molecular markers on a target on one side, and a second coating for binding to other devices on the opposite side.

10. A microstructural device of claim 9, wherein said second coating binds specifically to said first coating.

11. A method of making a microstructural device for identification and binding of specific molecular markers, comprising

depositing a sacrificial layer on a substrate,

generating at least one layer of a structural material on said sacrificial layer,

lithographically patterning said structural layer with desired sizes and shapes of a structure for said device, wherein the structure has at least one dimension greater than 0.2 microns,

etching said structural layer through to the sacrificial layer according to the lithographic pattern,

dissolving said sacrificial layer to release the device structures from the substrate, and

coating at least a portion of said device structures with at least one molecular coating which binds to specific targeted molecular markers.

12. A method of claim 11, wherein said lithographic patterning is photolithographic patterning.

13. A method of claim 11, wherein said lithographic patterning is electron beam lithography.

14. A method of claim 11, wherein said lithographic patterning is X-ray lithography.

15. A method of any of claims 11-14, wherein said sacrificial layer is silicon dioxide, said structural layer is CVD polysilicon, and said binding coating comprises antibodies.

16. A method of any of claims 11-15, further comprising binding to said structure a second coating providing aqueous suspension properties.

17. A method of claim 16, wherein said sacrificial layer is silicon dioxide, said structural layer is CVD polysilicon, said binding coating comprises antibodies, and said second coating comprises long chain hydrophilic molecules.

18. A method of any of claims 11-17, further comprising molding the substrate to produce a desired topography prior to deposition of the sacrificial layer, such that the resulting structures have corresponding mechanical features.

19. A method of any of claims 11-18, wherein at least one of said layer of a structural material is X-ray detectable.

20. A method of any of claims 11-18, wherein at least one of said layer of a structural material is magnetic resonance imaging (MRI) detectable.

21. A method of any of claims 11-20 further comprising coating said device structures with a detectable marker.

22. A method of claim 21, wherein said detectable marker is radioactive.

23. A method of claim 21, wherein said detectable marker is a dye.

24. A method of claim 21, wherein said detectable marker is fluorescent.

25. A method of detecting the presence of a molecular marker in a biological sample, comprising

contacting the microstructural device of any of claims 1-10 with said biological sample,

separating bound from unbound devices, and

detecting said bound microstructural device,

wherein detection of bound device indicates the presence of said molecular marker.

26. A method of Claim 25, wherein said detecting is by X-ray radiation.

27. A method of Claim 25, wherein said detecting is by magnetic resonance imaging (MRI).

28. A method of Claim 25, wherein said detecting is by gamma-ray radiation or ultrasound.

29. A method of any of claims 25-28, wherein said biological sample is selected from the group consisting of blood, plasma, urine, cerebrospinal fluid, cells, and tissues.

30. A method of any of claims 25-29, wherein said separating of bound and unbound devices is by washing.

31. A method of detecting a molecular marker during a surgical procedure, comprising

contacting a suspension of a labeled device of any of claims 1-10 with tissue, and detecting the binding of said labeled device,

wherein detecting of bound device indicates the presence of said molecular marker.

32. A method of claim 31, wherein said tissue is tissue surrounding a visually-detectable tumor and said molecular marker is a tumor-specific marker.

33. A method of claim 32, wherein said tumor-specific marker is selected from the group consisting of tumor autocrine mobility factor (TAMF), tumor angiogenesis factor (TAF), tumor-associated glycoprotein, and pancarcinoma antigen (PCA).

34. A method of forming emboli in a blood microvessel of a tissue, comprising

administering to a subject a therapeutically-effective amount of a suspension of a first device of any of claims 1-10 having a molecular binding coating specific to a molecular marker expressed in said blood microvessel, wherein a plurality of devices bind to the blood vessel wall; and

administering a therapeutically-effective amount of a suspension of a second device of any of claims 1-10 having a molecular binding coat specific to a target molecule on said first device,

wherein said second devices bind to said first devices, and wherein an artificial emboli is formed restricting further blood flow to said tissue.

35. A method of claim 34, wherein said molecular marker is a tumor-specific marker.

36. A method of claim 35, wherein said tumor-specific marker is collagen IV.

37. A therapeutic method of any of claims 34-36, wherein said effective amount of said first device suspension and second device suspension are each between 30 to 200 mls of a 5 % to 50 % suspension (volume/volume).

38. A suspension of microdevices for use in targeting selected target cells or tissue with a diagnostic agent, comprising

a suspension of bioerodable microdevices in an aqueous medium, said microdevices (i) having a selected non-spherical shape and uniform microdevice dimensions and (ii) containing said diagnostic agent.

39. The suspension of claim 38, wherein said microdevices have surface-bound, marker-binding molecules effective to bind to a marker carried on the surface of such target cells or tissue.

40. The suspension of claims 38 or 39, for use in targeting selected cells or tissue via the bloodstream, wherein said microdevices are coated with a hydrophilic polymer effective to enhance maintenance of the microdevices in suspension.

41. The suspension of claim 40, wherein said hydrophilic polymer is a natural or synthetic glycocalyx.

42. The suspension of claims 40 or 41, wherein said hydrophilic polymer is conjugated to vesicle-forming lipids, and said microdevices are coated with a lipid film containing such vesicle-forming lipids.

43. The suspension of any of claims 40-42, wherein said microdevices contain a marker-binding molecule (i) bound to the free ends of at least a portion of said polymer, and (ii) effective to bind to a marker carried on the surface of such target cells or tissue.

44. The suspension of any of claims 38-43, for use in parenteral administration to a subject, wherein said microdevices have a selected maximum dimension in the range between 0.1 and 3 microns.

45. The suspension of claim 44, for use in solid-tumor targeting, wherein said microdevices have a maximum dimension less than about 150 nm.

46. The suspension of any of claims 38-45, wherein the diagnostic agent is released from said microdevices upon microdevice bioerosion.

47. The suspension of any of claims 38-44, wherein said microdevices are formed of a material designed to dissolve in body fluid at a selected dissolution rate.

48. The suspension of claim 47, wherein said microdevices are formed of a material selected from the group consisting of iron, titanium, gold, silver, platinum, copper, and alloys and oxides thereof.

49. The suspension of any of claims 38-47, wherein said microdevices are formed of a biodegradable polymer material.

50. The suspension of any of claims 38-49, wherein the microdevices are substantially disc-shaped, and have a laminated structure containing first and second disc-shaped layers formed of first and second different materials, respectively.

51. The suspension of claim 50, wherein the first and second layers have different densities, and a combined density similar to that of the aqueous medium.

52. The suspension of claim 51, for use in parenteral administration to a subject, wherein the first and second layers have different rates of erosion in body fluid plasma.

53. The suspension of any of claims 38-52, wherein the microdevices have a trilaminate structure composed of an interior layer sandwiched between a pair of exterior coating layers, and the coating layers have a slower rate of bioerosion than the interior layer.

54. The suspension of any of claims 38-53, for use in detecting the presence of target cells in a subject, by intravenous injection of the suspension to the microdevices, wherein the microdevices are effective to circulate over an extended period in the bloodstream, and contain a diagnostic agent effective for detection by X-radiation, scintigraphy, nuclear magnetic resonance, or ultrasound.

55. The suspension of any of claims 38-54, for use in detecting the presence of target cells in a subject, by intraoperative administration of the suspension to the microdevices, wherein the microdevices contain a diagnostic agent effective for detection by X-radiation, scintigraphy, nuclear magnetic resonance, or ultrasound.

56. A microfabrication method for producing microdevices for use in the suspension of any of claims 38-55, comprising

forming a sacrificial layer on a substrate layer,

forming a microdevice-structure layer on said sacrificial layer,

lithographically patterning said microdevice-structure layer with a desired microdevice size and shape,

etching said microdevice-structure layer through to the sacrificial layer according to the lithographic pattern, and

dissolving said sacrificial layer to release the microdevices from the substrate.

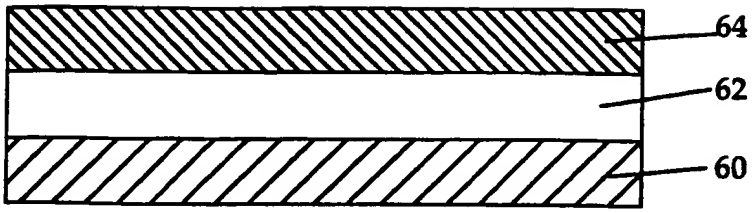


Fig. 1

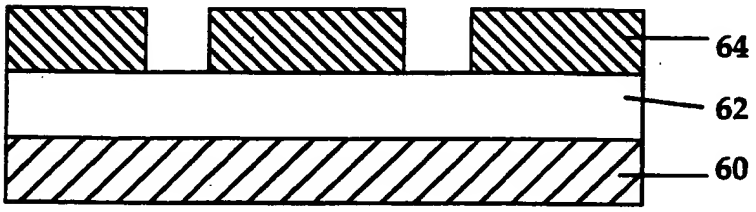
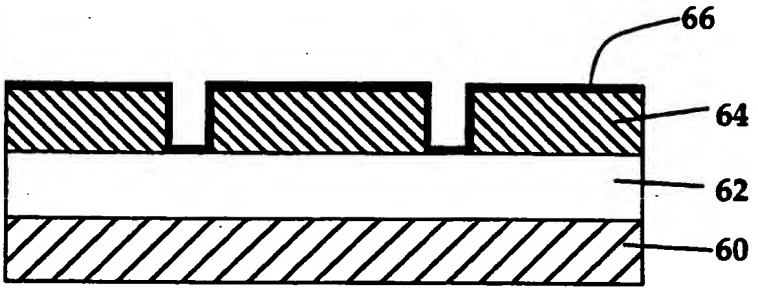


Fig. 2



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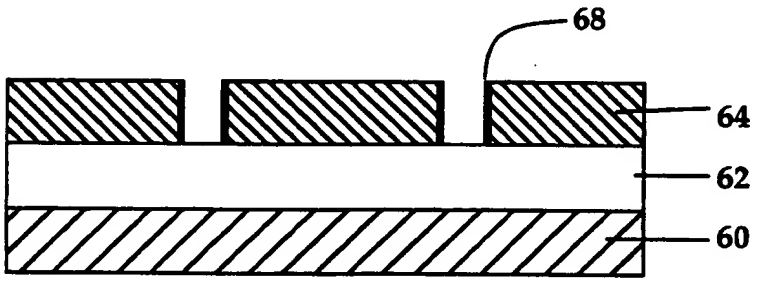

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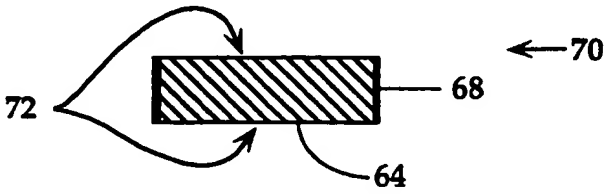

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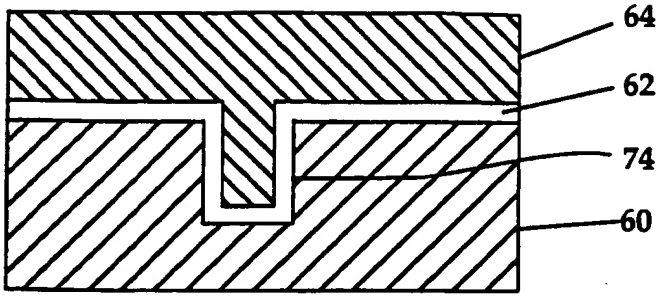

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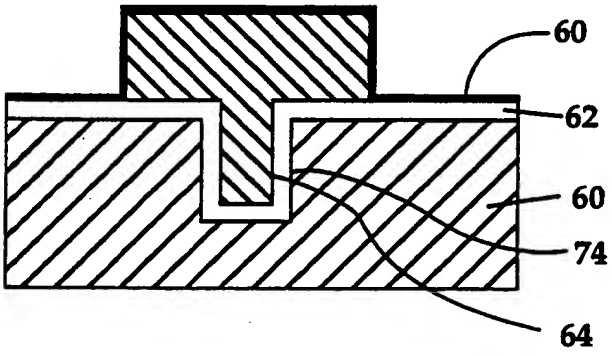

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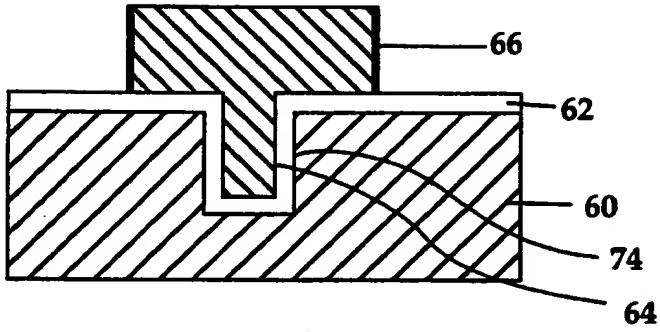

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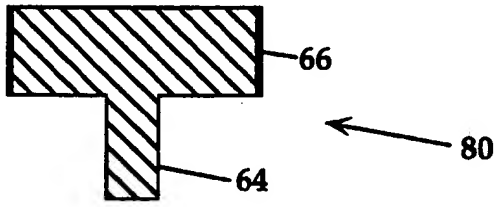

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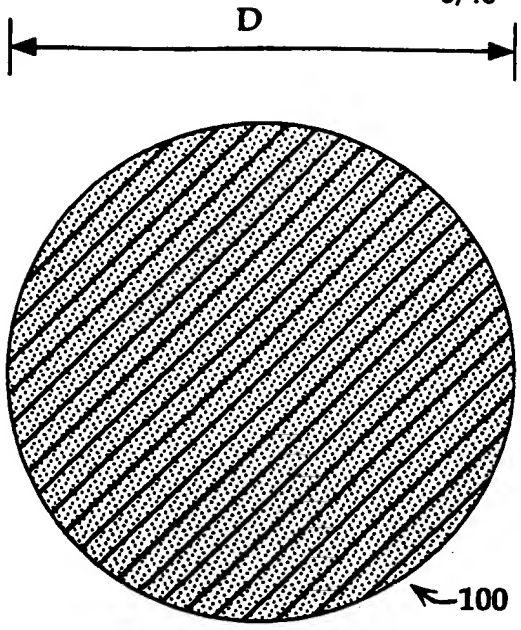

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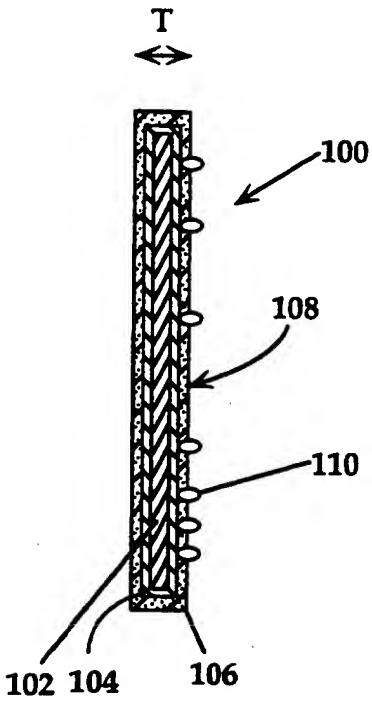

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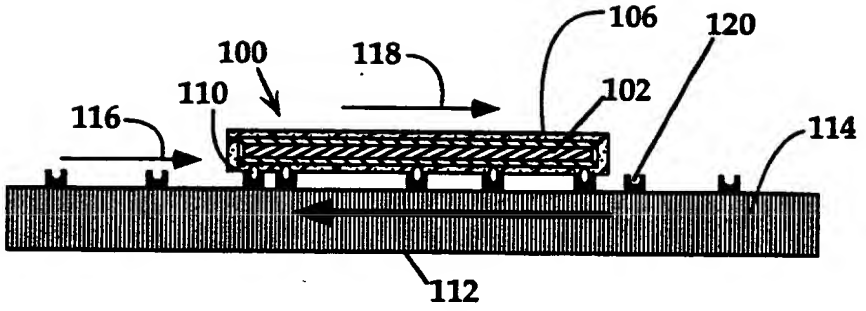

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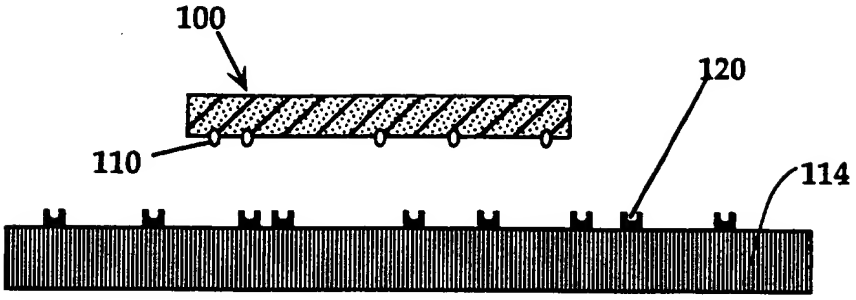
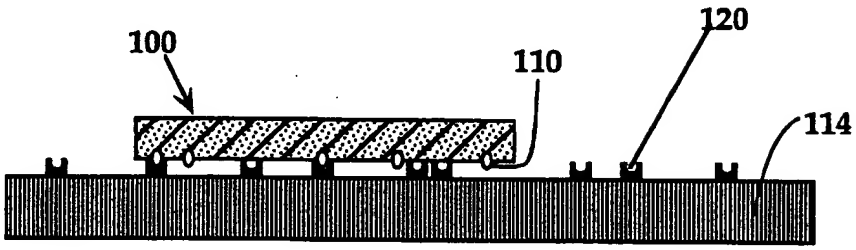
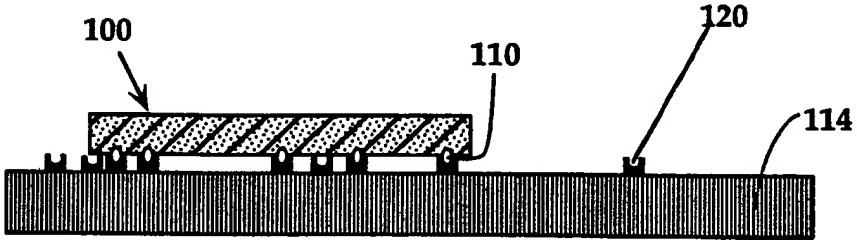


Fig. 13

Fig. 14





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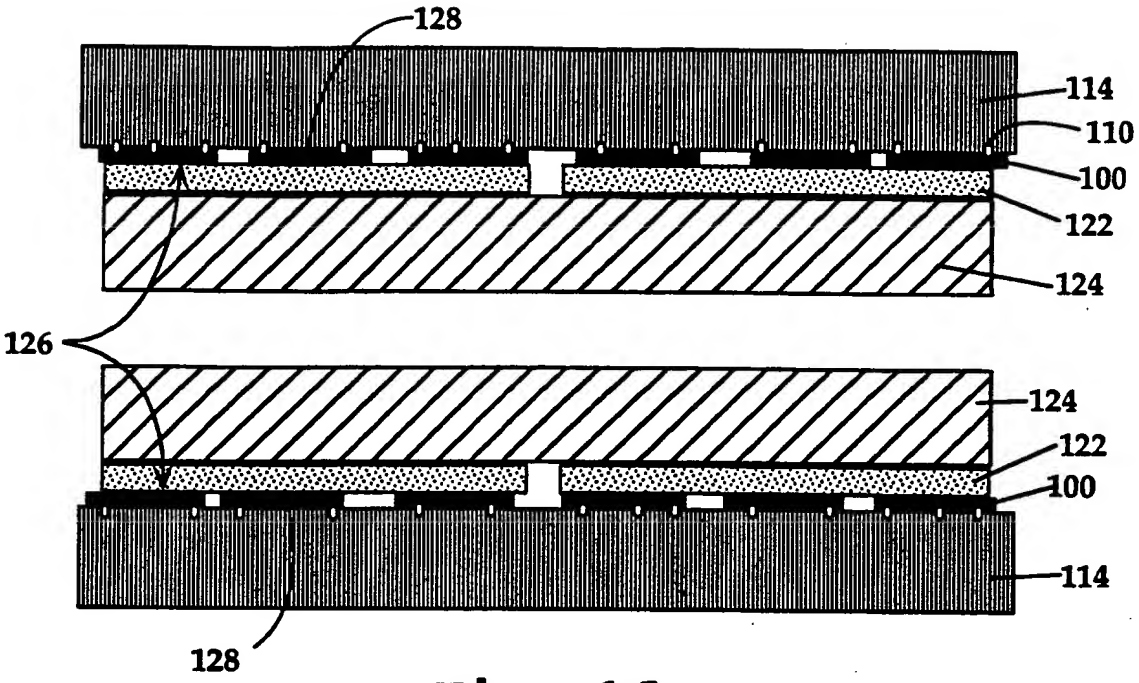
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Fig. 16

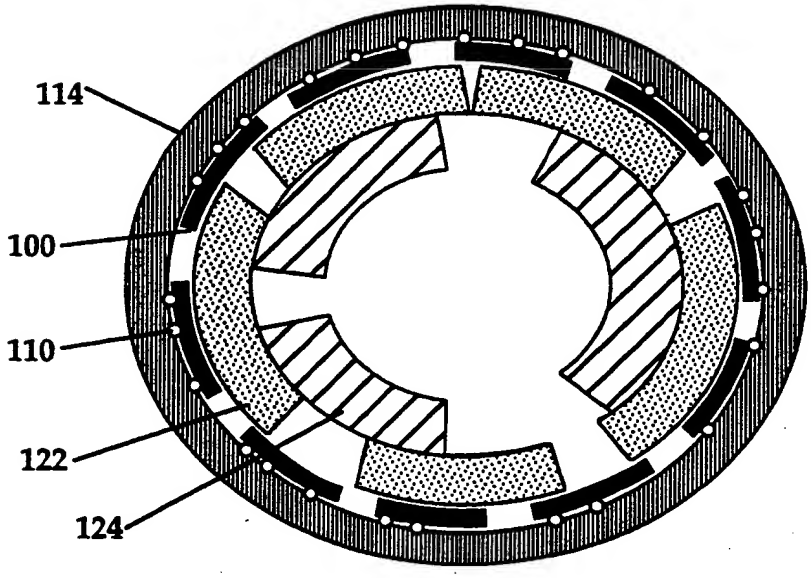
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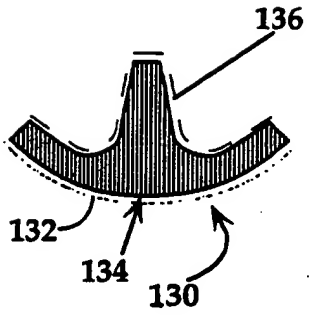
Fig. 17



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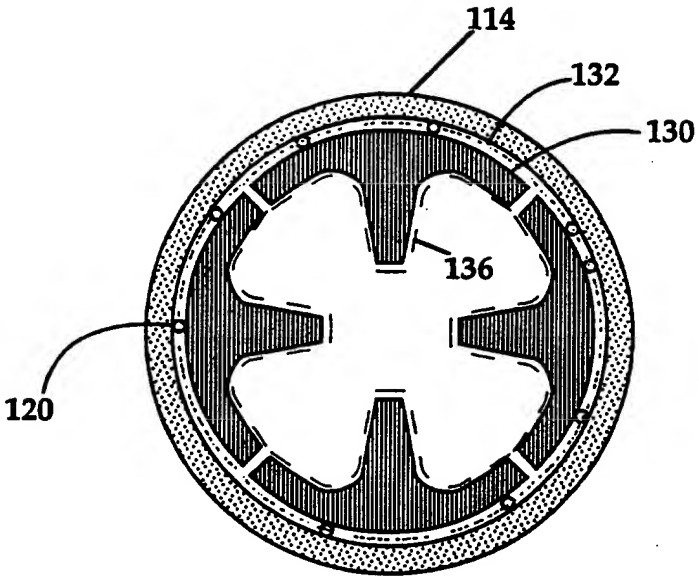

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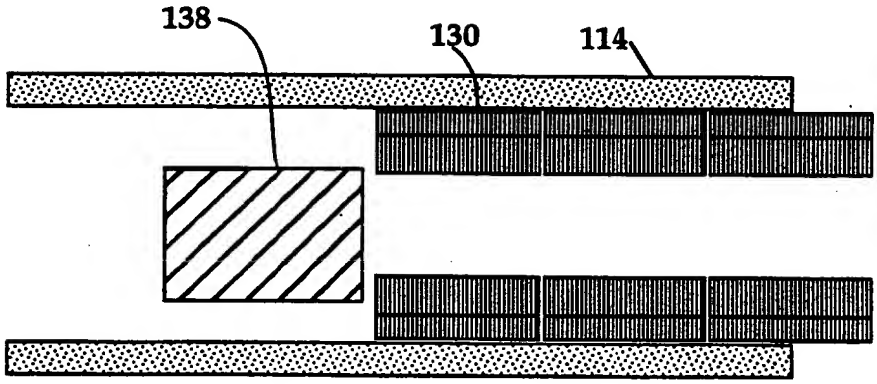

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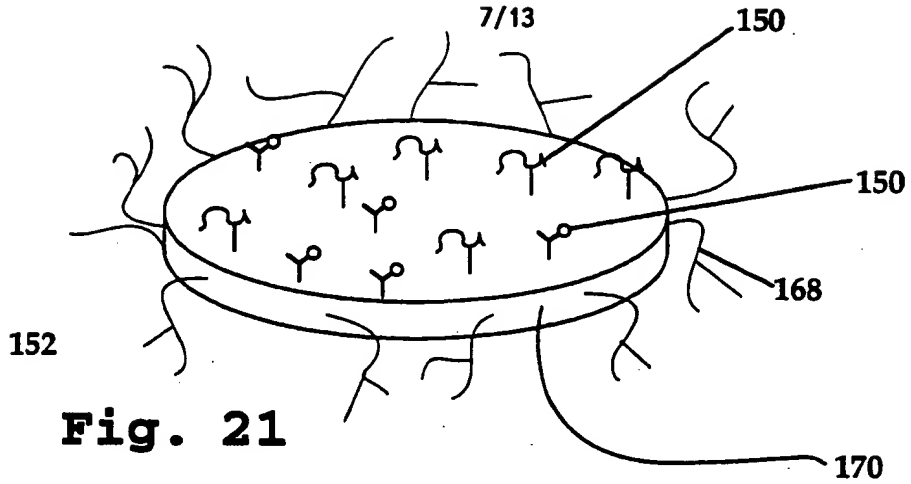

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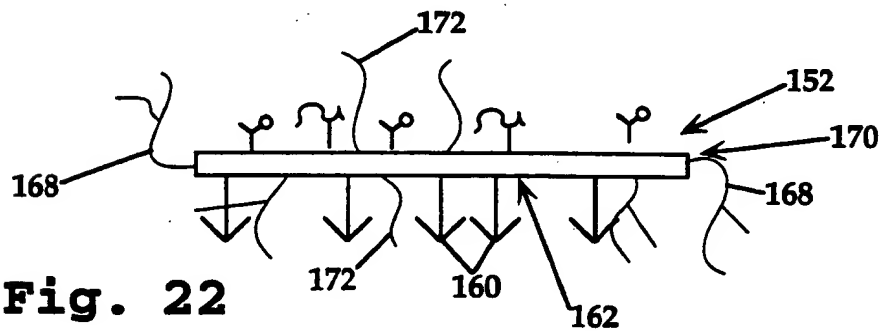

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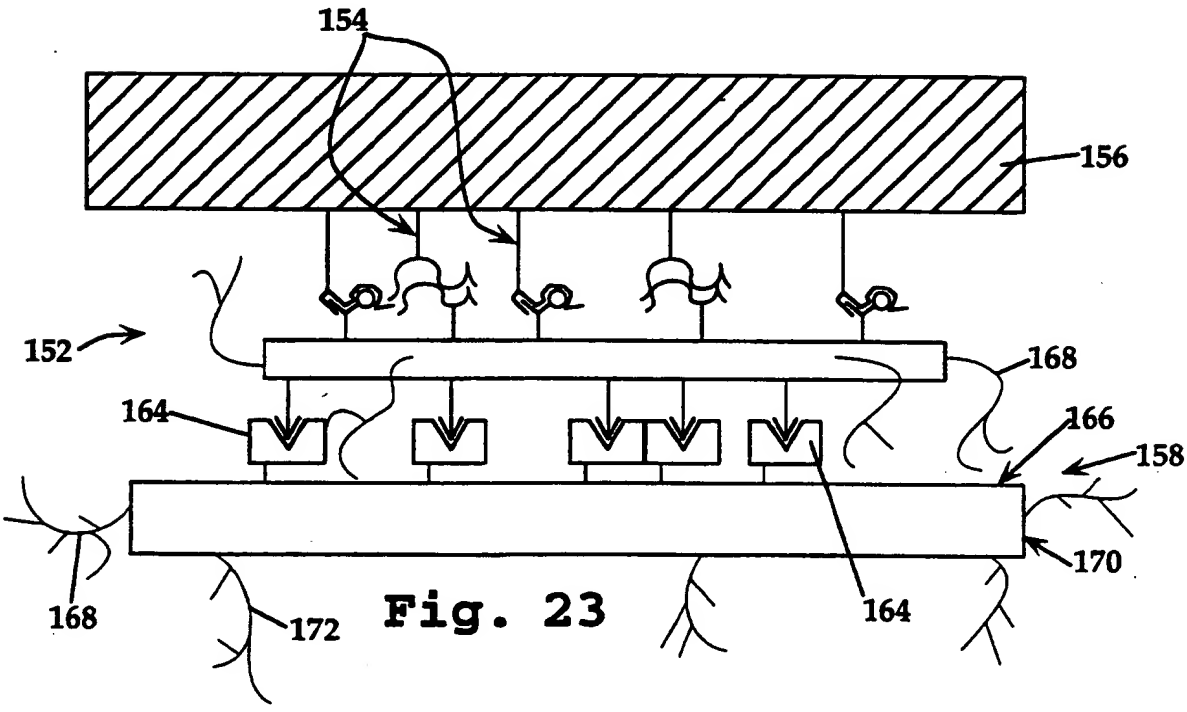

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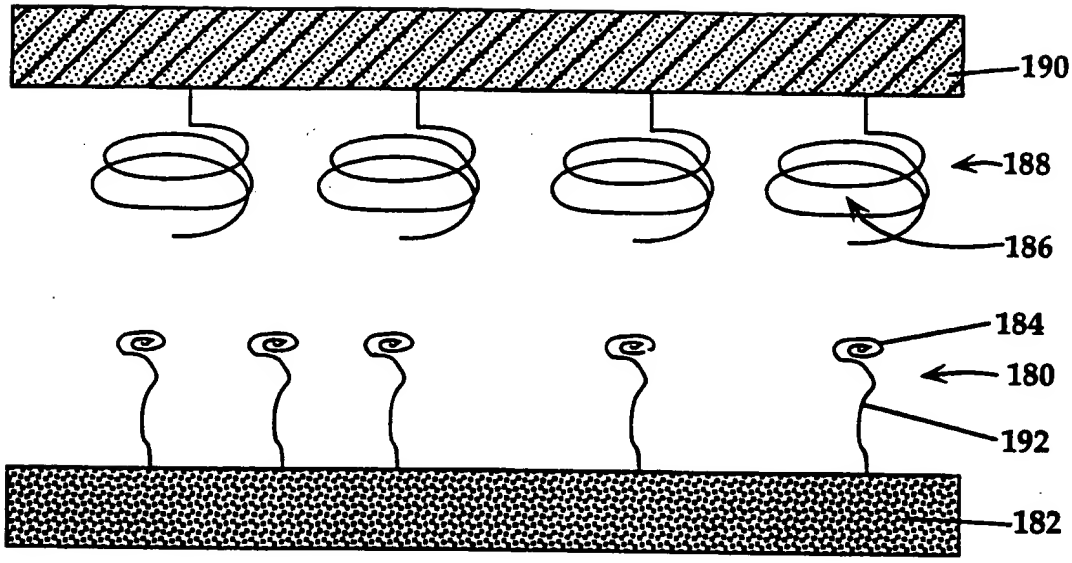

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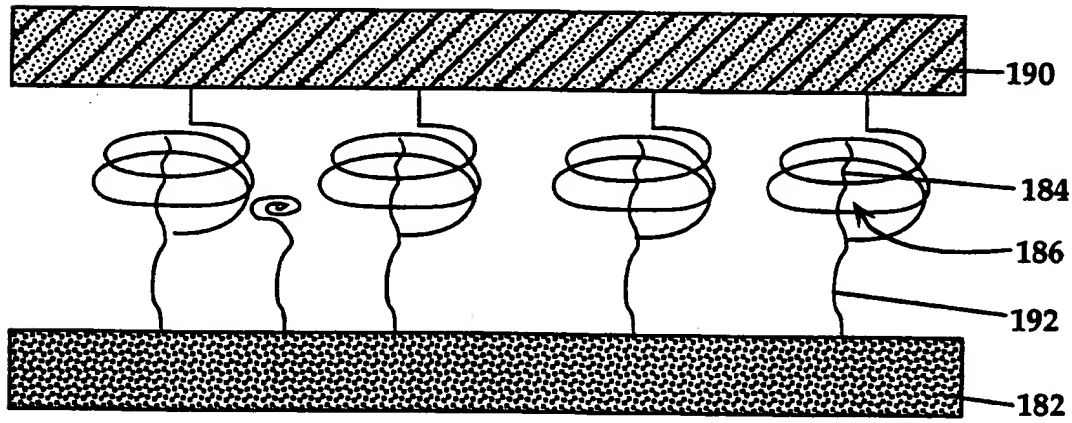

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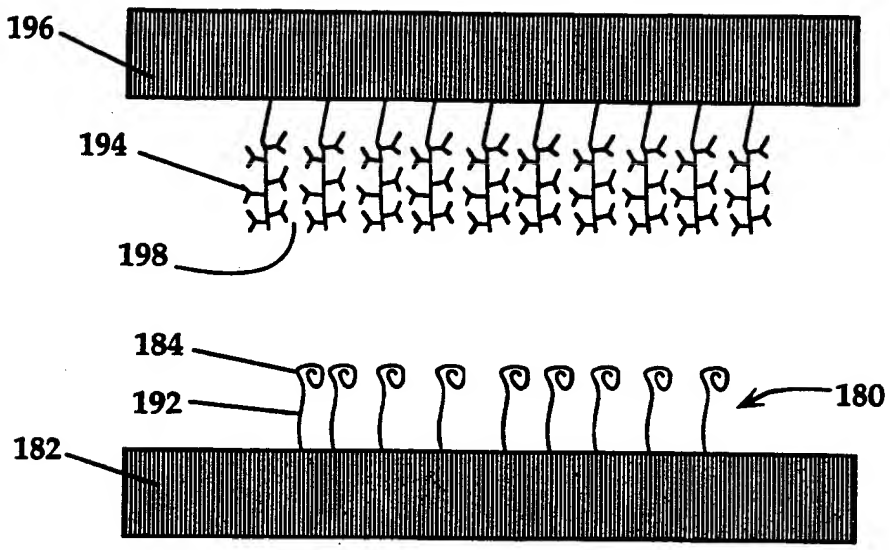

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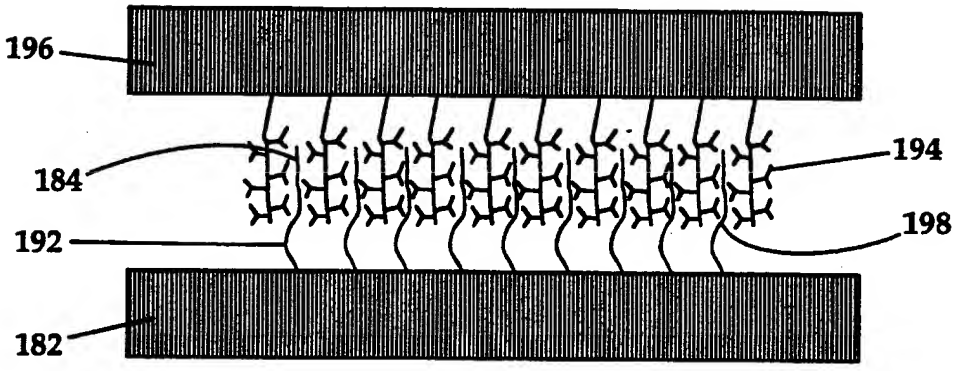

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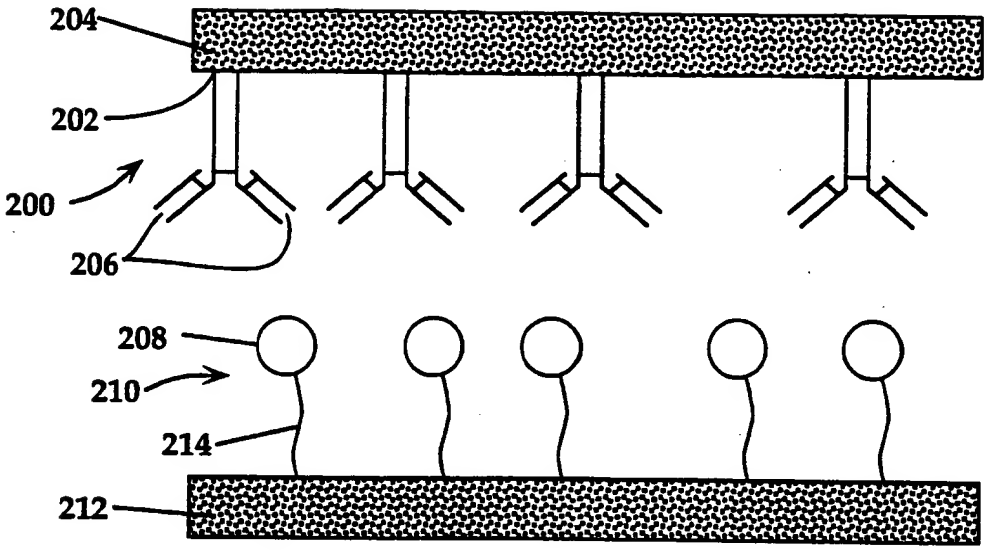

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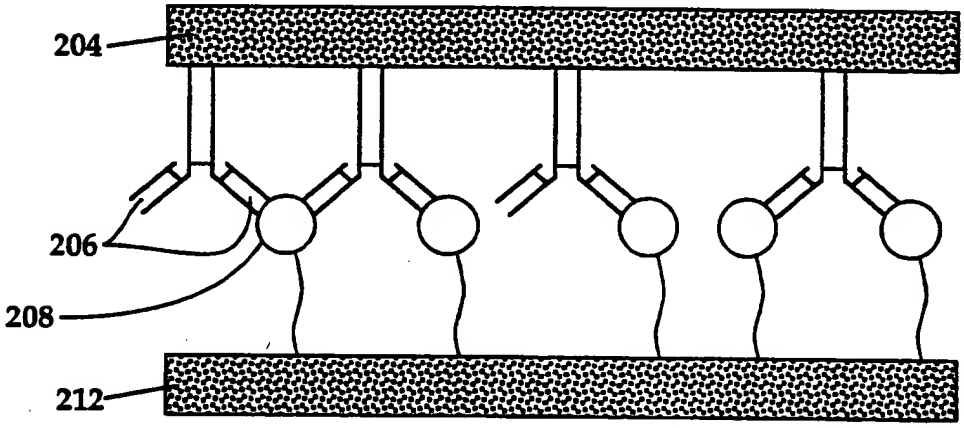

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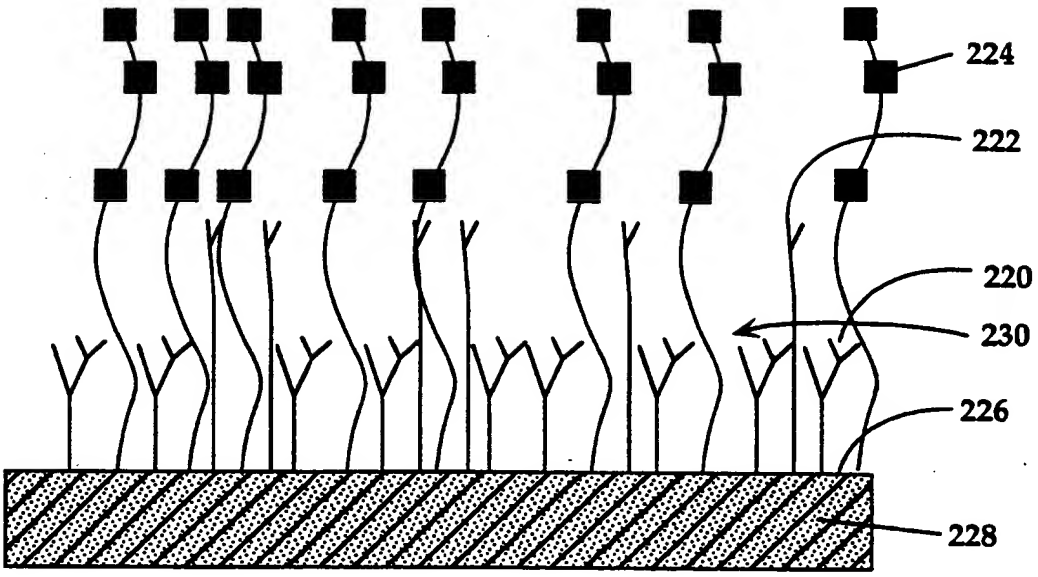

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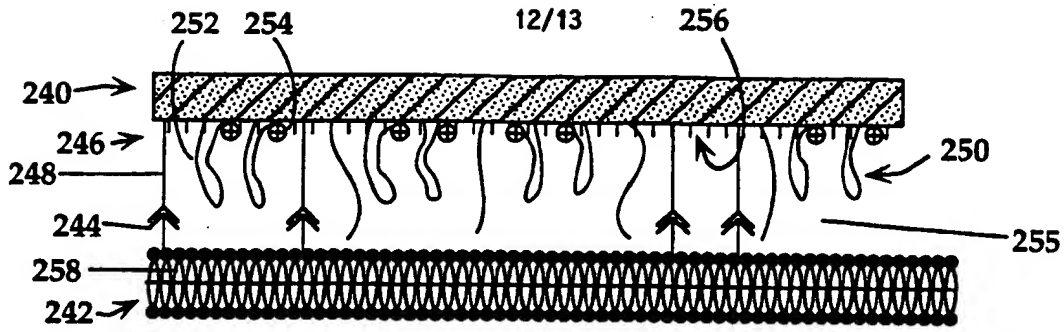

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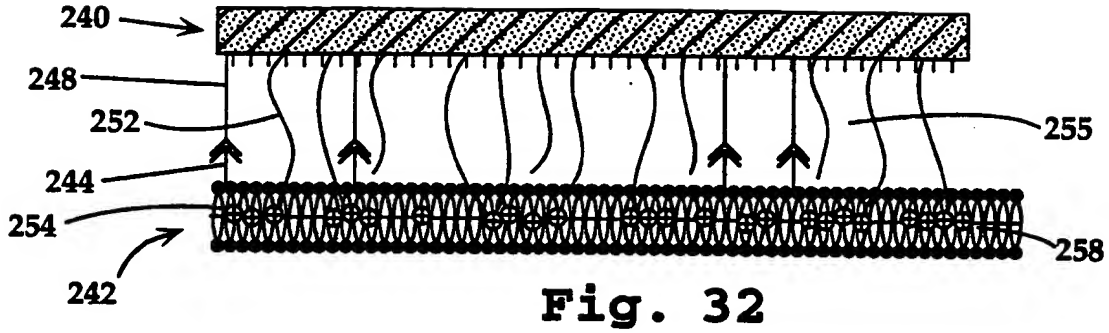

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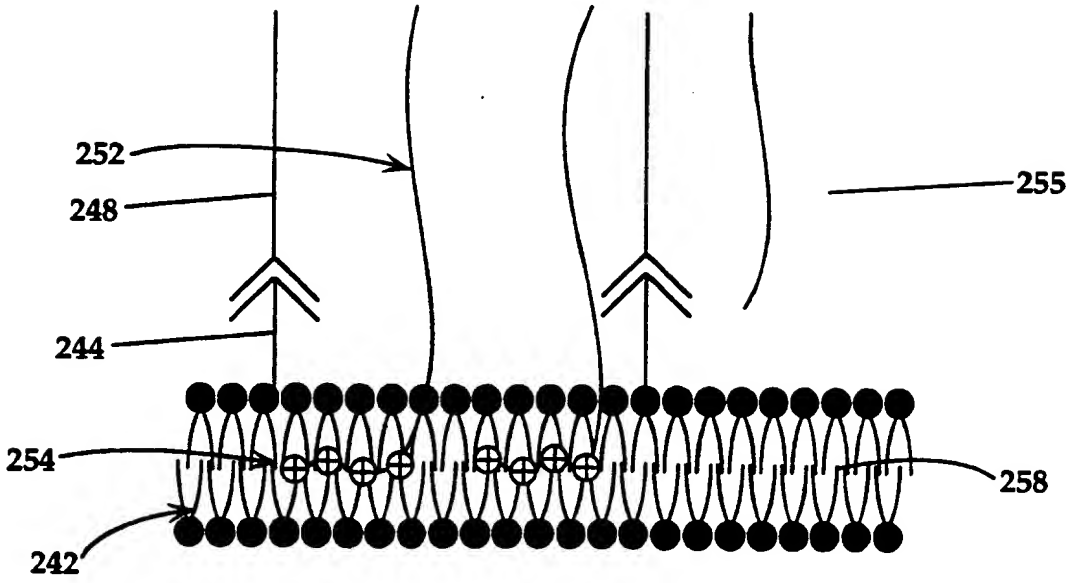

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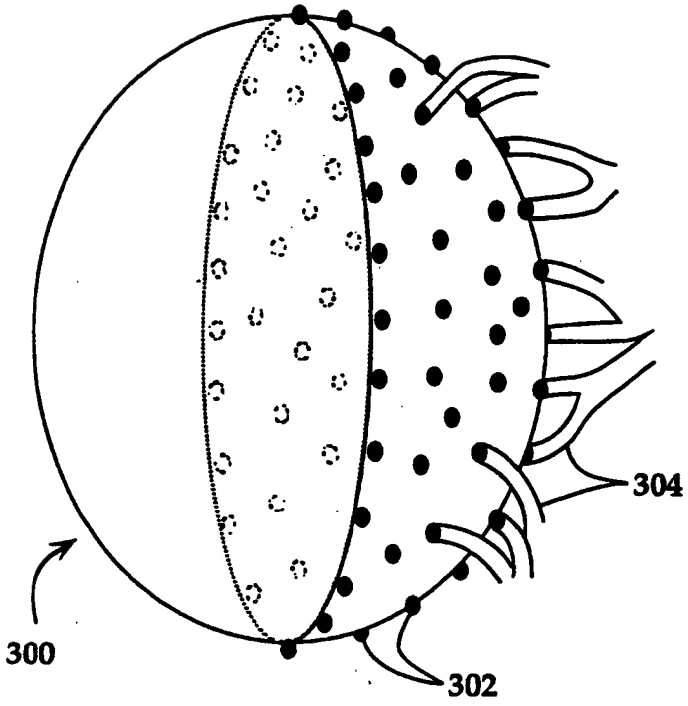

Fig. 34

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/09670

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61B 6/00
US CL :128/654

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 128/654, 662.02; 424/1.11, 1.17, 1.21, 1.29, 1.49, 9.1, 9.4

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 4,361,544 (GOLDENBERG) 30 november 1982, see entire document.	1-56
A	US, A, 3,927,193 (HANSEN ET AL.) 16 December 1975.	1-56

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 30 SEPTEMBER 1996	Date of mailing of the international search report 28 OCT 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3590	Authorized officer BRIAN CASLER Telephone No. (703) 308-3552

Form PCT/ISA/210 (second sheet)(July 1992)*